

Fluctuation in the Surface Morphology of DNA-wrapped Carbon Nanotubes Studied by Continuous Cross-sectional Analysis of Atomic Force Microscopy Image

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Abstract

Continuous cross-sectional analysis of atomic force microscope (AFM) images of several types of hybrids of DNA and carbon nanotubes (CNT) has been demonstrated. When conventional cross-sectional analysis was employed, the difference in surface roughness between chemical vapor deposition (CVD)-produced single-walled nanotubes (SWNTs) and high-pressure carbon monoxide (HiPco)-produced SWNT could not be distinguished. However, when continuous cross-sectional analysis was carried out using homemade software, greater fluctuation in surface roughness of DNA-HiPco SWNT hybrids was clearly quantified. Although hybrids of DNA-CNT have been studied intensively, fluctuation of surface morphology has not been well studied yet. Our results give useful information to understand the structures of DNA-CNT hybrids.

Keywords

Carbon Nanotube; DNA; Atomic Force Microscopy; Cross-Section; Binarization; Surface Morphology

Introduction

Hybridization of DNA and carbon nanotubes (CNT) has been intensively studied since the hybridization phenomenon was discovered in 2003 (Zheng *et al.*, 2003; Nakashima *et al.*, 2003). Hybridization was originally proposed as a method to disperse insoluble CNT by wrapping CNT molecules with water-soluble DNA. Further, because DNA can be designed variously, the DNA-CNT hybrids are expected to apply to medical bio devices and drug delivery (Kam *et al.*, 2005; Xu *et al.*, 2007; Kawaguchi *et al.*, 2011).

Although DNA-CNT hybrids have been studied by many researchers, interactions between DNA and CNT are not completely understood yet (Strano *et al.*,

2004; Kawamoto *et al.*, 2006; Tu *et al.*, 2009; Yarotski *et al.*, 2009; Khripin *et al.*, 2009; Yamamoto *et al.*, 2010). As an example, while the surface morphologies of DNA-CNT hybrids are not always uniform, the reason for this phenomenon is not clear (Karachevtsev *et al.*, 2008). To explain this, Lahiji *et al.* speculated that some parts of the CNT were not covered with DNA (Lahiji *et al.*, 2007). Further, Cathcart *et al.* suggested a possible model in which double-stranded DNA (dsDNA) split into single-stranded DNA (ssDNA) when DNA was adsorbed onto the CNT surface (Cathcart *et al.*, 2007). On the other hand, chiral structures of the CNT might be also important factors to determine manner of DNA adsorption.

To evaluate the surface morphology of DNA-CNT hybrids, atomic force microscopy (AFM) is a powerful method because the three-dimensional surface morphologies of samples are easily obtained. The thicknesses of DNA-CNT hybrids have been examined by several authors (Toita *et al.*, 2008; Yang *et al.*, 2009; Koh *et al.*, 2008). From those measurements, it was determined that DNA molecules were basically adsorbed onto CNT as monolayers. However, in all the cases, the cross-sectional AFM images were obtained at one point of one hybrid. Thus, fluctuation in the surface morphologies of the DNA-CNT hybrids has not been well studied yet. In this work, we examined continuous cross sections of DNA-CNT hybrids using homemade software in order to evaluate fluctuations in surface morphologies of DNA-CNT hybrids. CNT produced by different synthesis methods, ssDNA, and dsDNA were employed to compare various combinations. With our approach, fluctuations of the hybrids were quantitatively obtained.

Materials and Methods

Hybrids of DNA and bare single-walled carbon nanotubes (SWNT): single-stranded DNA (ssDNA, (dT)₃₀, Life Technologies Japan, Ltd., Tokyo, Japan) and double-stranded DNA (dsDNA, deoxyribonucleic acid sodium salt from salmon testes, Sigma-Aldrich Co., MO, USA) were dissolved in buffer solution containing 10 mM Tris-HCl and 1 mM EDTA (pH: 8.0). The dsDNA was sonicated for 60 min to obtain a uniform DNA solution. SWNTs produced by the high-pressure carbon monoxide (HiPco) method and by chemical vapor deposition (CVD) were purchased from Unidym, Inc., (CA, USA) and SES Research (TX, USA), respectively, as purified powders. For hybridization, 1 ml of DNA (ssDNA or dsDNA) solution (0.2 mg/ml) and 1 mg of SWNT powder (HiPco or CVD) were mixed, and then sonicated for 90 min (VCX 130, Sonics & Materials, Inc., CT, USA). The hybrid suspension was centrifuged at 14,100 g for 6 h. After centrifugation, the upper 80% of the volume was stored as the DNA-SWNT hybrid suspension.

Hybrids of dsDNA and water-soluble SWNT: The above-mentioned dsDNA solution was employed. A functionalized SWNT with polyethylene glycol (PEG SWNT) was purchased from Carbon Solutions, Inc. (Riverside, CA). A 1-mg/ml PEG SWNT suspension was prepared by sonication. The PEG SWNT suspension and dsDNA were mixed at a weight ratio of 5:6. After centrifugation at 18,500 g, the supernatant was removed for AFM observation.

AFM observation: For AFM samples, the suspension was diluted to the optimal concentration prior to dropping 10 μ l of the suspension onto an aminated mica surface. The samples were rinsed with water and then dried. AFM images were obtained in air in the AC mode (JSPM-5200, JEOL, Tokyo, Japan).

Results and Discussions

Figure 1 shows typical AFM images of DNA-CNT hybrids. In all the images, DNA-SWNT hybrids prepared with a small amount of DNA were clearly observed. Although smooth surfaces on the hybrids were observed in the dsDNA-CVD (a) and ssDNA-CVD (b) samples, dsDNA-HiPco (c) and ssDNA-HiPco (d) samples showed rough surfaces. Although conventional cross-sectional analysis of these samples has been reported in our previous paper, the fluctuation of the HiPco samples could not be well quantified by conventional cross-sectional analysis (Hayashida and Umemura, 2013). In Fig. 1, cross

sections of DNA-SWNT hybrids obtained from each AFM image are indicated. However, the cross sections shown in Figs. 1(c) and (d) were similar to those shown in Figs. 1(a) and (b). Thus, it was thought that continuous cross-sectional analysis would be necessary to quantify the roughness of the hybrids. By this approach, rough surfaces can be digitized as large deviation of the width values.

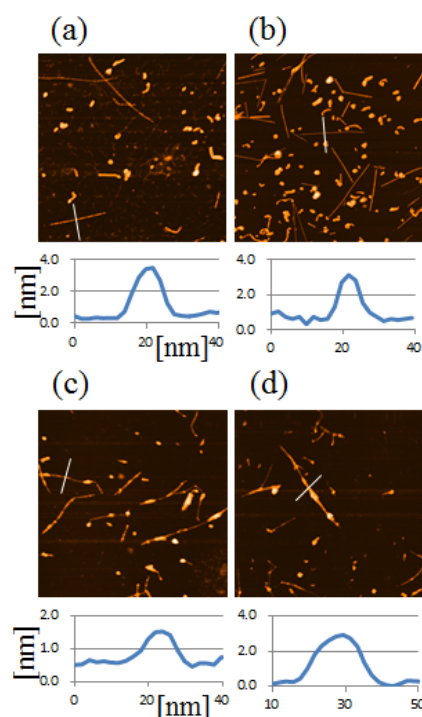


FIG. 1 AFM IMAGES AND CROSS SECTIONS OF DNA-SWNT HYBRIDS. (a) DSDNA-CVD, (b) SSDNA-CVD, (c) DSDNA-HIPCO, AND (d) SSDNA-HIPCO. SCAN SIZE WAS 2 M $\times$ 2 M.

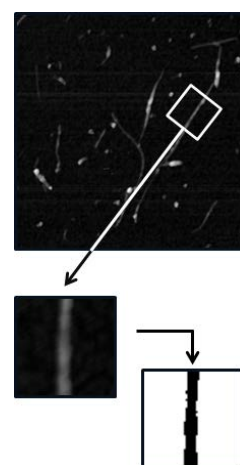


FIG. 2 SCHEMATIC VIEW OF CONTINUOUS CROSS-SECTIONAL ANALYSIS

Figure 2 shows a schematic view of the continuous cross-sectional analysis by the homemade software. From an AFM image, a target hybrid of DNA-CNT was cropped on a computer display and then binarized. Several threshold values for binarization

were examined, and optimal condition was fully utilized that correctly reflected edge of the observed objects. Subsequently, the number of black pixels (the area of the hybrid) was counted along every lateral line. Moreover, the size of a pixel could be calibrated from the original AFM image. Using the calibration, the width of the observed hybrids could be obtained.

Figure 3 shows the typical results of the continuous cross-sectional analysis described above. In the case of CVD SWNT, very flat features were obtained in both dsDNA and ssDNA. Although minute bumps and depressions were recognized on whole surfaces, it was difficult to judge whether the bumps and depressions were due to scanning noise or structural features. Because theoretical thickness of dsDNA is 2 nm as a single-layer, apparent width of the hybrids maybe was larger than 10 nm. However, the width obtained from the cross section of an AFM image is affected by the AFM tip shape, so the absolute value of the obtained width is meaningless. Therefore, we focused on the standard deviation of the width. Table 1 lists the average width of 10 hybrids with standard deviations. Relative standard deviations of dsDNA-CVD and ssDNA-CVD hybrids were 14% and 16%, respectively.

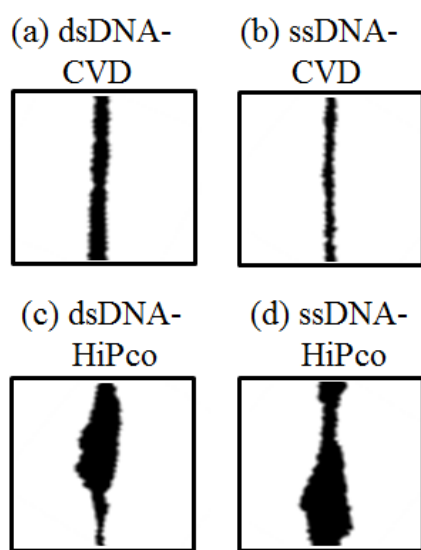


FIG. 3 WIDTH OF DNA-SWNT HYBRIDS OBTAINED BY CONTINUOUS CROSS-SECTIONAL ANALYSIS. (a) DSDNA-CVD, (b) SSDNA-CVD, (c) DSDNA-HIPCO, AND (d) SSDNA-HIPCO

TABLE. 1 AVERAGED WIDTH OF FOUR TYPES OF DNA-SWNT HYBRIDS OBTAINED BY CONTINUOUS CROSS-SECTIONAL ANALYSIS

Type of hybrids	Averaged width
dsDNA-CVD	20.2 ± 2.86 nm
ssDNA-CVD	13.6 ± 2.24 nm
dsDNA-HiPco	23.4 ± 6.57 nm
ssDNA-HiPco	25.0 ± 9.41 nm

However, in the case of the HiPco SWNT, large fluctuations were observed, which can be seen in Figs. 3(c) and (d). According to the quantitative data listed in Table 1, the standard deviations were 28% and 38% in dsDNA-HiPco and ssDNA-HiPco, respectively. Thus, the fluctuation of the hybrid surface observed in the AFM images was quantitatively discussed. As to comparison of dsDNA and ssDNA, large deviation was obtained in dsDNA in the case of CVD SWNT. On the other hand, the deviation of ssDNA was larger than that of dsDNA in the case of HiPco SWNT. The results suggested that difference of thickness of dsDNA and ssDNA was hidden in the large deviation in the HiPco SWNT.

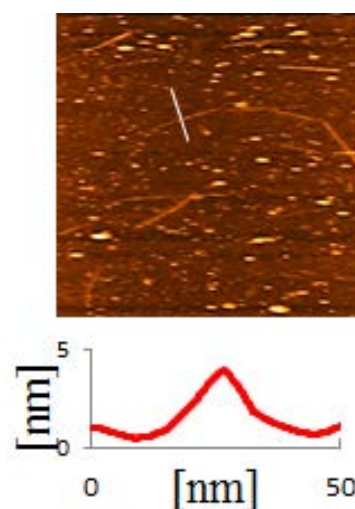


FIG. 4 AN AFM IMAGE AND THE CROSS SECTION OF DSDNA-PEG SWNT HYBRIDS. SCAN SIZE WAS $2 \text{ M} \times 2 \text{ M}$.

This analysis was applied to a water-soluble SWNT. Figure 4 shows a typical AFM image of dsDNA-PEG SWNT. In general, when DNA and SWNT are hybridized, the mixture of DNA and SWNT is sonicated to induce the hybridization. However, because PEG SWNT is water soluble, sonication is not applied to the samples. DNA solution was simply mixed with PEG SWNT solution. Although the sample was not sonicated, DNA-PEG SWNT hybrids were well fabricated. The surface of the hybrids was flat in the AFM image. When the fluctuation of the surface structures was evaluated by continuous cross-sectional analysis, the standard deviation of the width of the hybrids was found to be 15%.

Conclusions

The quantitative comparison of fluctuations in surface morphology of DNA-SWNT hybrids has been demonstrated. Continuous cross-sectional analysis using homemade software successfully calculated the

average widths and standard deviations in width on one DNA-SWNT hybrid. This approach may provide supportive information for a conventional cross-sectional analysis with one line.

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